



Histopathological evaluation of seven Amazon species of freshwater ornamental armored catfish

Rodrigo Yudi Fujimoto^{1*}, Mikaelle de Souza Neves², Rudã Fernandes Brandão Santos³, Claudinei da Cruz⁴, Daniel Guerreiro Diniz⁵ and Jorge da Costa Eiras⁶

¹Embrapa Tabuleiros Costeiros, Av. Beira Mar, 3250, Cx. Postal 44, 49025-040, Aracaju, Sergipe, Brazil. ²Universidade Federal do Pará, Belém, Pará, Brazil. ³Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil. ⁴Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista "Júlio de Mesquita Filho", Jaboticabal, São Paulo, Brazil. ⁵Universidade Federal do Pará, Instituto de Ciências Biológicas, Laboratório de Investigações em Neurodegeneração e Infecção, Hospital Universitário João de Barros Barreto, Belém, Pará, Brazil. ⁶Departamento de Biologia, Faculdade de Ciências, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal. *Author for correspondence. E-mail: ryfujim@hotmail.com

ABSTRACT. Fish commonly known as acaris or plecos are freshwater armored catfish economically important as a food resource and as ornamental fish. Most of these species are captured in the Amazon region. However, despite its economic importance, there is a lack of knowledge about their biological aspects. Thus, this study aimed to characterize and evaluate the histopathological aspects of important organs as gills, liver, integument and kidney of seven species of armored freshwater ornamental catfish from Guamá River, Pará State, Brazil. All organs showed typical characteristics of organs of other teleosts. In some species, gills and liver showed slight histopathological changes: telangiectasis, edema and morphological changes related to the presence of parasites (Monogenea and Digenea) in the gills, and changes in the arrangement of hepatocytes rows, and vacuolation of hepatocytes in the liver. Thus, the knowledge of the normal structure of organs and changes found can be used as tools for environmental and health monitoring of animals.

Keywords: fish, environmental quality, Acaris, histology.

Avaliação histopatológica em sete espécies de cascudos ornamentais amazônicos

RESUMO. Os peixes conhecidos como Acaris ou Cascudos são siluriformes de água doce com o corpo revestido por placas ósseas, economicamente importantes como fonte de alimento e peixes ornamentais. A maioria destas espécies é capturada na região amazônica. Contudo, apesar de sua importância econômica, há desconhecimento sobre os aspectos biológicos desses peixes. Assim, o objetivo deste trabalho foi caracterizar e avaliar histologicamente órgãos importantes (brânquias, fígado, rim e tegumento) de sete espécies de peixes ornamentais de água doce do rio Guamá, Pará, Brasil. Todos os órgãos observados apresentaram as características típicas dos órgãos de teleósteos. Em algumas espécies, as brânquias e o fígado mostraram pequenas alterações histopatológicas: edema, telangiectasias e alterações morfológicas relacionadas com a presença de parasitas (Monogenea e Digenea) nas brânquias, e modificações no arranjo de linhas de cordões de hepatócitos e vacuolização destas células foram também observadas. Assim, o conhecimento da estrutura dos órgãos e as alterações encontradas podem ser utilizados como ferramentas para o monitoramento ambiental e sanitário dos animais.

Palavras-chave: peixe, qualidade ambiental, Acaris, histologia.

Introduction

In the Guamá River basin, Northeastern Pará State, Brazil, the capture of ornamental freshwater fish, namely *Corydoras* spp. and armored catfish commonly known as plecos, is the main economic activity for many riverine families (SANTOS et al., 2012) inserido a data na referencia). Fish are captured using several nets and/or by hand while free diving, and stocked in small net containers in the river until sale. During this period, specimens are usually subjected to several adverse

environmental conditions derived mainly from domestic effluents, which can cause poor water quality and stress.

The influence of these adverse environmental conditions may produce changes in several fish organs, mainly in gills, integument, liver and kidney. Therefore, the knowledge of the histological structure of these organs may indicate the general health condition of fish (ALBINATI et al., 2009; DIAZ et al., 2003; MACHADO; FANTA, 2003; SANTOS et al., 2004), and contribute to

monitoring the environmental quality and eventual adverse effects of treatments and handling of these specimens in export facilities.

Histopathological analysis represent a useful tool for the diagnosis of several fish diseases, and this is demonstrated in several textbooks (GENTEN et al., 2009) or book chapters (REIMSCHUESSEL, 2008; ROBERTS; RODGER, 2004) dealing with fish histology and histopathology.

Gills are probably the most important organ to be observed due to their respiratory function and delicate structure (MELETTI et al., 2003). This organ is highly sensitive to even small changes in environmental conditions, presence of pollutants, toxic compounds, pathogenic organisms, etc. (CANLI; ATLI, 2003; SANTOS et al., 2012), which cause more or less intense alterations in the gill epithelium easily detected by routine histological observations. Therefore this organ is considered a good indicator of environmental conditions (SCHWAIGAR et al., 1997).

The histological examination of the organs associated with the digestive tract, mainly the liver, is important to understand the general condition of fish due to their importance for digestion and the possibility to accumulate several substances, as well as its role against intoxication (AU, 2004; CRUZ et al., 2005; LEMES; BRACCINI, 2004).

Kidney is another key organ with an important role for homeostasis (BANKS, 1992; FUJIMOTO et al., 2008; TAKASHIMA; HIBIYA, 1995), and Schwaiger et al. (1997) consider this organ as indicator of the environmental quality assessed by histological studies. According to Takashima and Hibiya (1995), the exposure to some chemicals, or in the case of excessive blood within the organ, pathological changes may occur in the Bowman capsule, as well as abnormal proliferation of epithelial cells and thickening of the basal membrane.

Finally, the integument that represents the first barrier to invaders through the production of mucus and associated substances (FAST et al., 2002; FLETCHER, 1978). It is composed by two layers, the epidermis and the dermis. The histology of the integument, for instance, the number and size of mucous and chloride cells may constitute an indicator of a pathological process (LEE et al., 1996).

Almost nothing is known about these aspects concerning freshwater ornamental armored catfish. Thus, this study aimed to characterize and evaluate

the histopathological aspects of important organs as gills, liver, integument and kidney of seven species of armored freshwater ornamental catfish from Guamá river, Pará State, Brazil.

Material and methods

Seven species were captured *Lasiancistrus saetiger* (Armbruster, 2005) (common name: acari canoa n = 10), *Peckoltia oligospila* (Günther, 1864) (acari bola, n = 10), *Rineloricaria cf. lanceolata* (acari loricaria, n = 10), *Leporacanthicus galaxias* Isbrücker and Nijssen, 1989 (acari pinima, n = 10), *Cochilodon* sp. (acari pleco, n = 10), *Hypostomus* sp. (acari picoto, n = 10) and *Ancistrus* sp. (acari ancistrus, n = 10).

After sampling in the Guamá River, Pará State, specimens were killed by anaesthetic overdose and samples of gills (first gill arch), liver, kidney and integument (ventral portion) were collected. Samples were fixed in 10% buffered formalin, routinely processed for histology and embedded in Paraplast. Histological sections were stained with hematoxylin and eosin (BEHMER et al., 1976).

Results and discussion

Gill morphology was normal, formed by gill arches, primary and secondary lamellae. The secondary lamellae were lined by a thin layer of epithelial cells that covered the pillar cells.

Chloride cells (Figure 1C), acidophilic and intensely stained, were observed in all species. These cells are common in marine fish and are involved in the osmotic regulation (FISCHER-SCHERL; HOFFMANN, 1988). They have been observed in freshwater fish like *Ictalurus punctatus* (GRIZZLE; ROGERS, 1985) and *Oreochromis niloticus* (GARCIA-SANTOS et al., 2007) having a prominent role in acid-base balance.

In the gills of some specimens, different pathological characteristics were observed: telangiectasia (Figure 2A) and edema (Figure 2B). These changes in the gill structure may be caused by several factors as decrease of water quality (AZIM; LITTLE, 2008), and may also be a response of gills to the presence of heavy metals like cadmium (GARCIA-SANTOS et al., 2007; LIAO et al., 2007). Furthermore, the presence of parasites in the gills (monogenoidea and encysted digenetic metacercariae) was observed (Figure 2C and D, respectively) causing the displacement of the secondary gill lamellae.

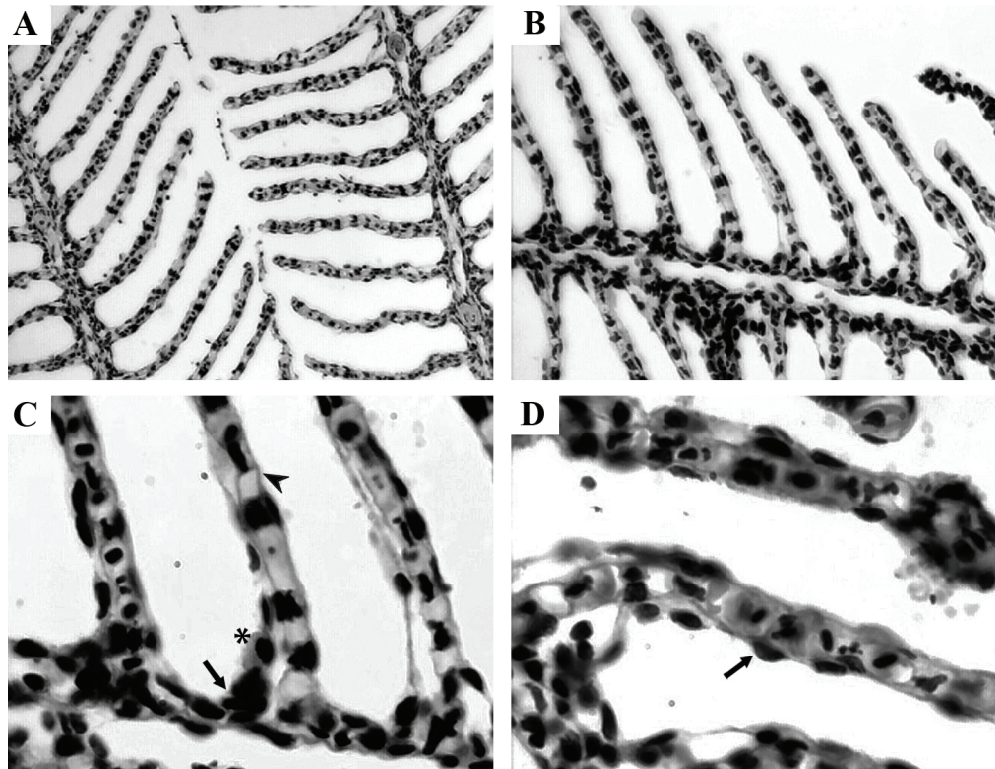


Figure 1. Photomicrograph of gills of several species of armored fish from the Guamá River (A, *Hypostomus* sp., B, C, *Ancistrus* sp., D, *Cochilodon* sp.). Note the secondary lamellae in A and B, mucous cell (asterisk in C), chloride cell (arrow in C), and pillar cell (triangle arrow in C). Epithelial cells are clearly observed in D (arrow). Magnifications: A, B, 200x, C, D, 1000x.

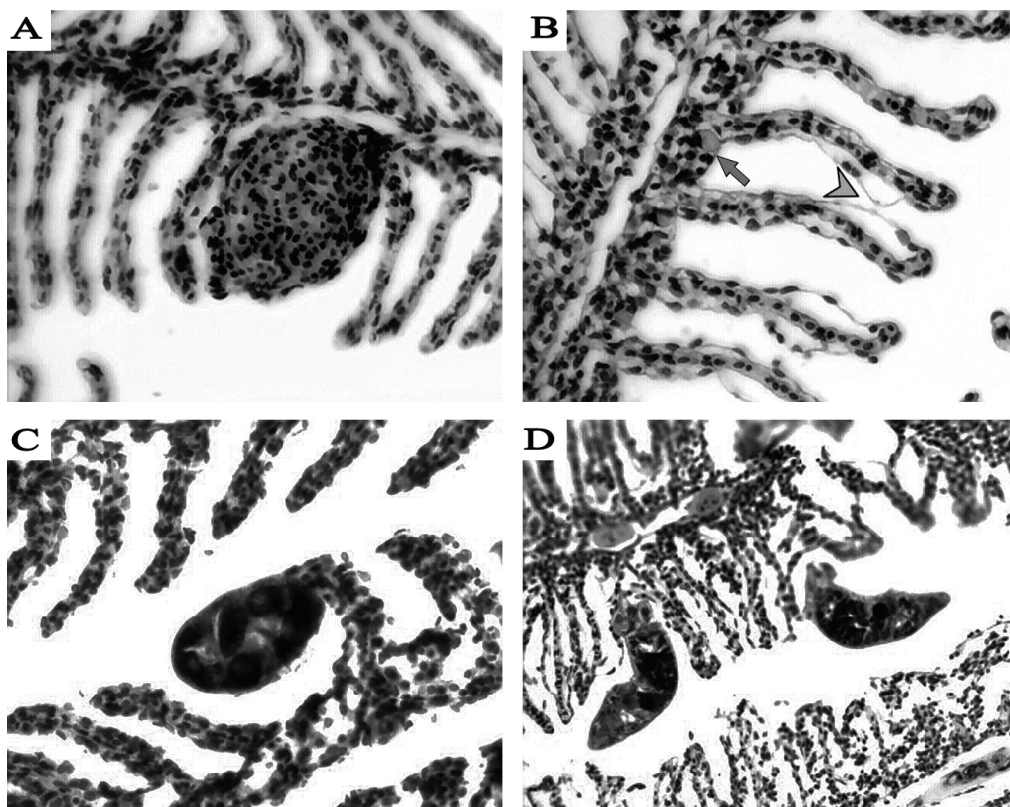


Figure 2. Photomicrograph of histopathological features in acaris gills: A, telangiectasis in *Ancistrus* sp.; B, edema (arrowhead) in *L. saetiger*, and chloride cell (arrow); C, encysted trematode in *Rineloricaria* cf. *lanceolata*; D, monogonoidea in *L. galaxias*. Magnification: 400x.

Gills are usually regarded as good indicators of the general environmental quality once the structure of the organ responds promptly to environmental degradation, frequently by hyperplasia of cells on the basis of the secondary lamellae, thus merging these structures, sometimes with dramatic consequences on the respiratory function. In the present study, the structure of the gills was normal and, in particular, the absence of cellular hyperplasia between the secondary lamellae is indicative of good environmental condition.

Histologically, liver of acaris has row-arranged hepatocytes. Sinusoidal capillaries, lined by endothelial cells with prominent nuclei, converge to the central vein, and are irregularly distributed between two rows of hepatocytes. Hepatocytes were round in shape with clear cytoplasm and central nuclei (Figure 3A and B). In the periphery of hepatic lobes, we observed a structure formed by a vein and one liver channel (Figure 3C and

D). According to Lemes and Braccini (2004), these structures are called not true triads once they have not exactly the same properties of mammalian triads. Our results agree with the general description of Grizzle and Rogers (1985) for *Ictalurus punctatus*. Peters et al. (1987) noted that despite the liver has different roles for the organisms, its structure is homogeneous and formed by a hepatic parenchyma with hepatocytes arranged in double rows with sinusoidal capillaries, as observed in armored catfish

Some histopathological features were observed in the liver of *Hypostomus* sp.: changes in the arrangement of hepatocytes rows, vacuolation of hepatocytes at varied levels, and reduced size of hepatocyte nuclei (Figure 4). According to Peters et al. (1987) features like cells hypertrophy, capillaries congestion, and fat deposition may occur in older fish. Nevertheless, these features may be the result of adverse environmental conditions. In our case, we could not determine the cause of the changes detected.

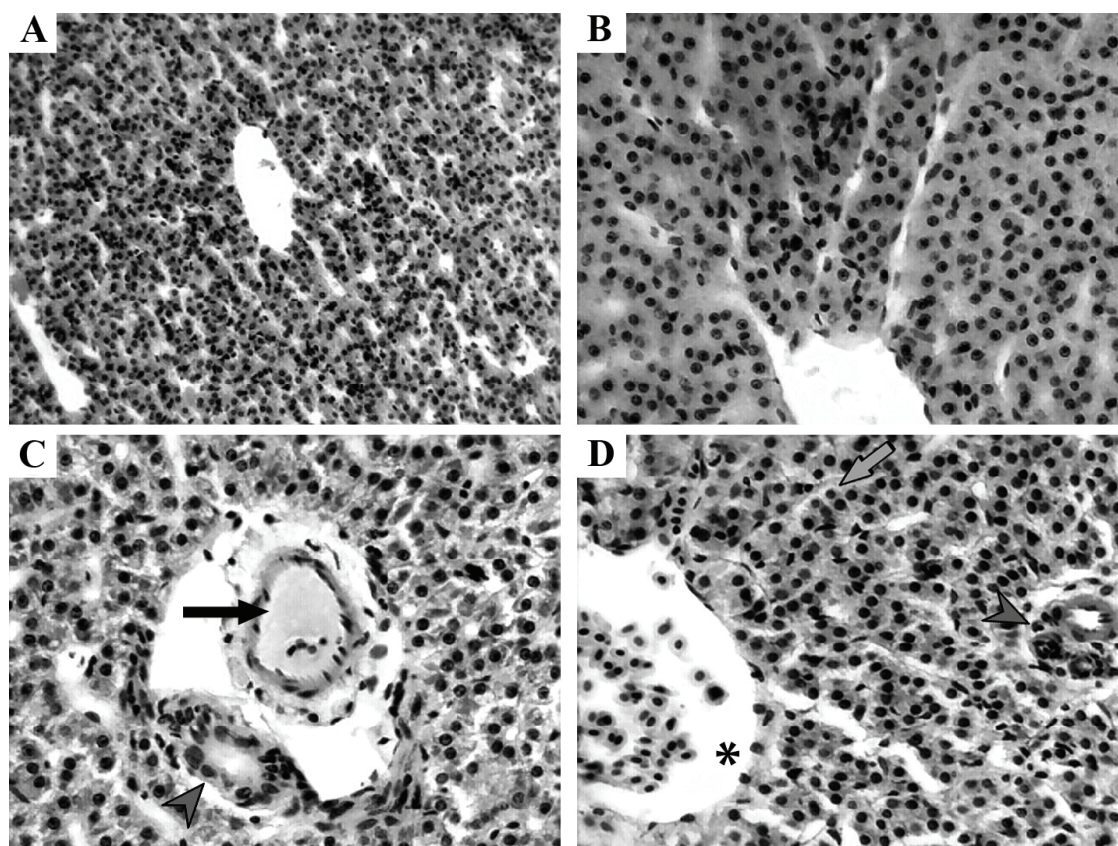


Figure 3. Photomicrographs of the liver of acaris from the Guamá River. A, Central vein of one lobe of the liver of *Ancistrus* sp.; B, Arrangement of hepatocytes in the liver of *L. saetiger*; C, Branch of the Portal vein (arrow) and branch of the bile duct (arrowhead) in the liver of *Rineloricaria* cf. *lanceolata*; D, vein in the center of the lobe (asterisk), portal triad (arrowhead) and row-arranged hepatocytes (arrow) in the liver of *Cochilodon* sp. Magnifications: 200x (A) and 400x (B-D).

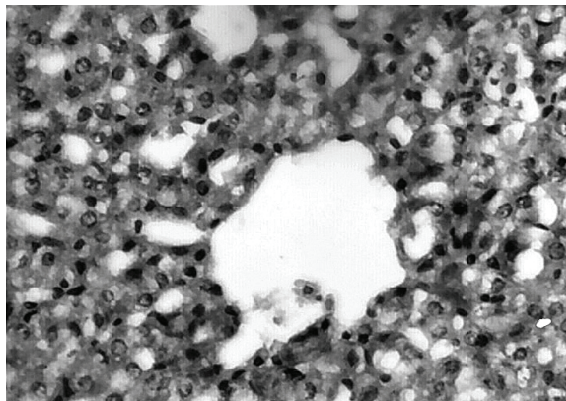


Figure 4. Modification of the row arrangement of hepatocytes, vacuolation of hepatocytes, and reduced size of nuclei of hepatocytes in the liver of *Hypostomus* sp. Magnification: 400x.

The ventral integument of the acaris is made up of a stratified epithelium with two layers: epidermis, the external one, and the dermis. Flattened cells and goblet cells are observed in the epidermis (Figure 5). The dermis consists of dense connective tissue. Between the epidermis and the dermis abundant deposition of melanin may occur, as is the case of *L. galaxias* (Figure 5B).

The integument participates in several important functions, such as exchange of gas, ions, molecules, secretion of mucous substances, osmoregulation, immunity, and forms a physical barrier against different aggressive agents (WAHLI et al., 2003). One of the most important roles of the integument is the secretion of mucus, which constitutes a defense against pathogens (FLETCHER, 1978), once it contains lysosomes and antibacterial activity (FAST et al., 2002).

Therefore, the study on integument morphology may be important to assess environmental stress, inadequate nutrition or intoxication. In agreement with Lee et al. (1996) changes in the number and size of mucous cells and chloride cells may be an indicator of environmentally induced pathogenesis. In our observations we did not observe such condition.

The kidney of acaris is constituted by kidney corpuscles formed by glomerulus and capillaries, which is covered by the Bowman's capsule formed by a double cell layer, the parietal and visceral epithelium. Between these two layers is found the Bowman space, which extends to the lumen of kidney tubules. Among the kidney corpuscles is placed the hematopoietic tissue (Figure 6).

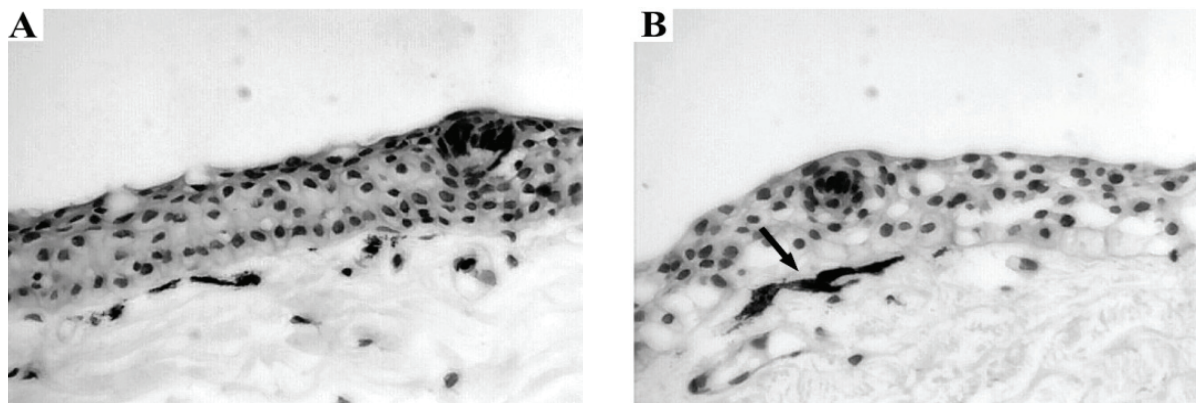


Figure 5. A, histological section of the integument of *L. saetiger* showing the epidermis, dermis, and mucous cells; B, integument of *L. galaxias* showing deposition of melanin (arrow). Magnification: 400x.

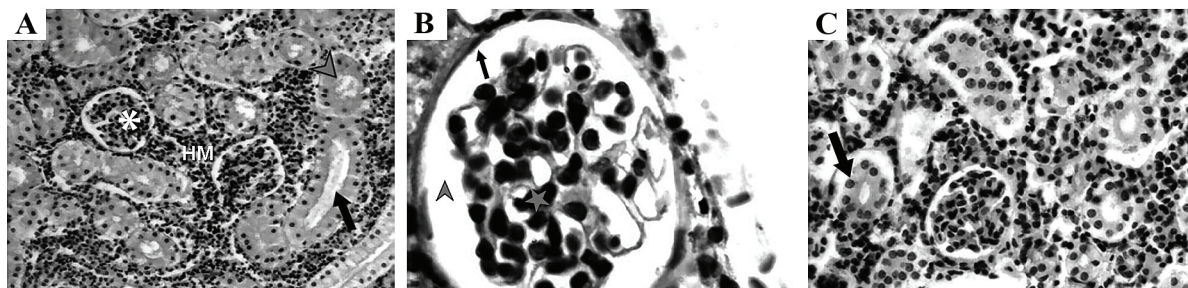


Figure 6. Photomicrograph of the kidney of three species of acaris. A, general structure of the kidney of *Hypostomus* sp.: glomerulus (star), hematopoietic tissue (HM), kidney tubules, first distal segment (arrow) and distal segment (arrowhead); B, detail of the glomerulus of *L. galaxias* depicting the capillaries (star), Bowman capsule (arrow) and Bowman space (arrowhead); C, detail of the second proximal segment (arrow) of a kidney tubule of *Rineloricaria* cf. *lanceolata*. Magnifications: A, 200x, B, C, 1,000x.

Kidney tubules of the acaris are divided in three different parts: the first proximal segment, the second proximal segment and the distal segment. The first proximal segment is formed by a columnar epithelium with the nuclei at the base of cells. The second proximal segment is thinner and the nuclei have a more or less central position within the cells. The distal segment has about the same size of the second proximal segment, but nuclei are at the base of cells (Figure 6). These features are similar to the descriptions of the kidney of other species like *Ictalurus punctatus* (GRIZZLE; ROGERS, 1985) and *Piaractus mesopotamicus* (MATAQUEIRO et al., 2009).

Conclusion

Acaris have histological characteristics concerning the gills, liver, integument and kidney similar to the features observed in other fish species. Some alterations were observed, and they may be related to environmental conditions or to specimens' age.

The knowledge of the normal structure of these organs, given in the present research, may be an important tool for environmental monitoring and disease diagnosis.

Acknowledgements

The participation of J.C. Eiras in this research was partially supported by the European Regional Development Fund (ERDF) through the COMPETE - Operational Competitiveness Programme and National Funds through FCT - Foundation for Science and Technology, under the project "PEst-C/MAR/LA0015/2013.

References

- ALBINATI, A. C. L.; MOREIRA, E. L. T.; ALBINATI, R. C. B.; CARVALHO, J. V.; DE LIRA, A. D.; SANTOS, G. B.; VIDAL, L. V. O. Biomarcadores histológicos - toxicidade crônica pelo Roundup em piaçu (*Leporinus macrocephalus*). **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 61, n. 3, p. 621-627, 2009.
- ARMBRUSTER, J. W. The loricariid catfish genus *Lasiancistrus* (Siluriformes) with descriptions of two new species. **Neotropical Ichthyology**, v. 3, n. 4, p. 549-569, 2005.
- AU, D. W. T. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. **Marine Pollution Bulletin**, v. 48, n. 9-10, p. 817-834, 2004.
- AZIM, M. E.; LITTLE, D. C. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). **Aquaculture**, v. 288, n. 1-4, p. 29-35, 2008.
- BANKS, W. J. **Histologia veterinária aplicada**. São Paulo: Manole, 1992.
- BEHMER, A. O.; TOLOSA, E. M. C.; FREITAS-NETO, A. G. **Manual de técnicas para histologia normal e patológica**. São Paulo: Edusp/Edart, 1976.
- CANLI, M.; ATLI, G. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. **Environmental Pollution**, v. 121, n. 1, p. 129-136, 2003.
- CRUZ, C.; FUJIMOTO, R. Y.; LUZ, R. K.; PORTELLA, M. C.; MARTINS, M. L. Toxicidade aguda e histopatologia do fígado de larvas de trairão (*Hoplias lacerdae*) expostas à solução aquosa de formaldeído a 10%. Pesticidas: **Revista de Ecotoxicologia e Meio Ambiente**, v. 15, p. 21-28, 2005.
- DIAZ, A. O.; GARCÍA, A. M.; DEVINCENTI, C. V.; GOLDEMBERG, A. L. Morphological and histochemical characterization of the mucosa of the digestive tract in *Engraulis anchoita*. **Anatomia, Histologia, Embryologia**, v. 32, n. 6, p. 341-346, 2003.
- FAST, M. D.; SIMS, D. E.; BURKA, J. F.; MUSTAFA, A.; ROSS, N. W. Skin morphology and humoral non-specific defense parameters of mucus and plasma in rainbow trout, coho and Atlantic salmon. **Comparative Biochemistry and Physiology Part A**, v. 132, n. 3, p. 645-657, 2002.
- FISCHER-SCHERL, T.; HOFFMANN, R. W. Gill morphology of native brown trout *Salmo trutta m. fario* experiencing acute and chronic acidification of a brook in BAVARIA, F. R. G. **Diseases of Aquatic Organisms**, v. 4, n. 4, p. 43-51, 1988.
- FLETCHER, T. C. Defense mechanism in fish. In: MALINS, D. C.; SARGENT, J. R. (Ed.). **Biochemical and biophysical perspectives in marine biology**. London: Academic Press, 1978. p. 189-222.
- FUJIMOTO, R. Y.; CRUZ, C.; MORAES, F. R.; Análise de efluente e histologia da pele, fígado e rim de pacus (*Piaractus mesopotamicus*) suplementados com cromo trivalente. **Boletim do Instituto de Pesca**, v. 34, n. 1, p. 117-124, 2008.
- GARCIA-SANTOS, S.; MONTEIRO, S. M.; CARROLA, J.; FONTAINHAS-FERNANDES, A. Alterações histológicas em brânquias de tilápia nilótica *Oreochromis niloticus* causadas pelo cádmio. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v. 59, n. 2, p. 376-381, 2007.
- GENTEN, F.; TERWINGHE, E.; DANGUY, A. **Atlas of fish histology**. Enfield: Science Publishers, 2009.
- GRIZZLE, J. M.; ROGERS, W. A. **Anatomy and histology of the channel catfish**. Auburn: Auburn University, 1985.
- GÜNTHER, A. Catalogue of the fishes in the British. **Museum**, v. 5, p. 1-455, 1864.
- LEE, T. H.; HWANG, P. P.; LIN, H. C. Morphological changes of integumental chloride cells to ambient cadmium during the earling development of teleost, *Oreochromis niloticus*. **Environmental Biology of Fishes**, v. 45, n. 1, p. 95-102, 1996.

- LEMES, A. S.; BRACCINI, M. C. Descrição e análise histológica das glândulas anexas do trato digestório de *Hoplias malabaricus* (Bloch, 1794), (Teleostei, Erythrinidae). **Biodiversidade Pampeana**, v. 2, n. 1, p. 33-41, 2004.
- LIAO, C.-Y.; ZHOU, Q.-F.; FU, J.-J.; SHI, J.-B.; YUAN, C.-G. Interaction of methylmercury and selenium on the bioaccumulation and histopathology in medaka (*Oryzias latipes*). **Environmental Toxicology**, v. 22, n. 1, p. 69-77, 2007.
- MACHADO, M. R.; FANTA, E. Effects of the organophosphorous methyl parathion on the branchial epithelium of a freshwater fish *Metynnis roosevelti*. **Brazilian Archives of Biology and Technology**, v. 46, n. 3, p. 361-372, 2003.
- MATAQUEIRO, M. I.; NAKAGHI, L. S. O.; SOUZA, J. P.; CRUZ, C.; OLIVEIRA, G. H. Urbinati, E. C. Histopathological changes in the gill, liver and kidney of pacu (*Piaractus mesopotamicus*, Holmberg, 1887) exposed to various concentrations of trichlorfon. **Journal of Applied Ichthyology**, v. 25, n. 1, p. 124-127, 2009.
- MELETTI, P.; ROCHA, O.; MARTINEZ, C. B. R. Avaliação da degradação ambiental na bacia do rio Mogi-Guaçu por meio de testes de toxicidade com sedimento e de análises histopatológicas em peixes. In: BRIGANTE, J.; ESPÍNDOLA, E. L. G. (Ed.). **Limnologia fluvial: um estudo no rio Mogi-Guaçu**. São Carlos: Rima Editora, 2003. p. 149-180.
- PETERS, N.; KOHLER, A. A.; KRANZ, H. Liver pathology in fishes from the Lower Elbe as a consequence of pollution. **Diseases of Aquatic Organisms**, v. 2, p. 87-97, 1987.
- REIMSCHUESSEL, R. General fish histopathology. In: EIRAS, J. C.; SEGNER, H.; WAHLI, T.; KAPOOR, B. G. (Ed.). **Fish diseases**. United States: Science Publishers, 2008. p. 1-40.
- ROBERTS, R. J.; RODGER, H. D. The pathophysiology and systematic pathology. In: ROBERTS, R. J. (Ed.). **Fish Pathology**. 3rd ed. London: Saunders, 2004. p. 62-143.
- SANTOS, A. A.; RANZANI-PAIVA, M. J. T.; FELIZARDO, N. N.; RODRIGUES, E. L. Análise histopatológica de fígado de tilápia-do-nilo, *Oreochromis niloticus*, criada em tanque-rede na represa de Guarapiranga, São Paulo, SP, Brasil. **Boletim do Instituto de Pesca**, v. 30, n. 2, p. 141-145, 2004.
- SANTOS, R. F. B.; DIAS, H. M.; FUJIMOTO, R. Y. Acute toxicity and histopathology in ornamental fish amazon blue spotted corydora (*Corydoras melanistius*) exposed to formalin. **Anais da Academia Brasileira de Ciências**, v. 84, n. 4, p. 1001-1007, 2012.
- SCHWAIGER, J.; ADAM, S.; PAWERT, M.; HONNEN, W.; TRIEBSKORN, R. The use of histopathological indicators to evaluate contaminant-related stress in fish. **Journal of Aquatic Ecosystem Stress and Recovery**, v. 6, n. 1, p. 75-86, 1997.
- TAKASHIMA, F.; HIBIYA, T. **Atlas of fish histology**. Normal and Pathological Features. Tokio: Kodansha, 1995.
- WAHLI, T.; VERLHAC, V.; GIRLING, P.; GABAUDAN, J.; AEBISCHER, C. Influence of dietary vitamin C on the wound healing process in rainbow trout (*Oncorhynchus mykiss*). **Aquaculture**, v. 225, n. 1-4, p. 371-386, 2003.

Received on November 17, 2013.

Accepted on April 23, 2014.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.